

Biological Properties of Iranian *Zataria Multiflora* Essential Oils: A Comparative Approach

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Abstract

Zataria multiflora Boiss, known as Avishan-e-Shirazi in Persian, with traditional pharmacological properties take advantage of being useful medicinal plant. In this study, the chemical composition of different parts of Iranian *Z. multiflora* Boiss oils and also its antioxidant and antibacterial properties have been considered. The results showed that the main oil components especially *thymol* and *carvacrol* are identical in the oils extracted derived from leaf, flower and aerial parts of the plant. In addition, the different part oils indicated the high radical scavenging and antioxidant properties. Also, *E. coli* and *P. aeruginosa* are more sensitive to the effects of the oil samples. However, the flower and aerial part oils of *Z. multiflora* are more efficient than the leaf in sensitivity to these microorganisms. These findings suggest the antioxidant and antibacterial activities of the oils derived from the leaf, flower and aerial parts of *Z. multiflora* regarding *thymol* and *carvacrol* as the major components. With a view to these biological properties, the applications of the oils in medicinal purposes are promising findings.

Keywords: *Zataria multiflora*; Essential oils; Antioxidant activity; Antibacterial activity.

1. Introduction

Zataria multiflora Boiss (Lamiaceae; Voucher Number: 41754) known as Avishan-e-Shirazi in Persian, is a thyme-like plant that grows wild in central and southern parts of Iran. It is an important flowering plant in Iranian traditional medicine. Several pharmacological properties such as antimicrobial, antifungal, anti-seizure, anti-nociceptive, anti-candida, anti-septic, anti aphthous, analgesic, carminative and anti-inflammatory effects have been reported for this plant. Flavonoids and essential oils are the most important components with reported pharmacological activities [1-10]. It has been reported that the *Z. multiflora* essential oils can stimulate innate immunity [11] and have antibacterial, antifungal

and antioxidant activities [12-15]. Moreover, the oil of *Z. multiflora* successfully inhibited the growth of bacteria associated with gastrointestinal infections, including *Staphylococcus aureus*, enterohemorrhagic *Escherichia coli*, *Salmonella Typhi* and *Paratyphi*, and *Shigella flexneri* and *Bacillus cereus* [16-18]. Its essential oil has also been proposed for disorders of respiratory and gastrointestinal system [19]. Moreover, the plant oil is a down-regulator of MDM2 gene expression which highlights the effectiveness of this oil in malignant disease [20].

To consider partly, the extensive uses of *Z. multiflora* in healthcare and medicine, in this study we investigated –for the first time– the chemical composition of different parts (i.e., leaf, flower and aerial parts) of Iranian *Z. multiflora* Boiss oil extracts. Moreover, the antioxidant and antibacterial properties of these oils extracted from three divisions have been compared.

2. Materials and Method

2.1 Plant materials

Different members of fresh *Z. multiflora* i.e. flower, leaf and aerial parts (leaf, flower, and stem) were collected separately in May 2010 from the Shiraz city, Iran. Dr. Younes Asri (Botanist) from herbarium of Iranian botanical garden (TARI) authenticated the plant materials with the voucher number 41754.

2.2 Oil extraction and analysis

Oil extraction from the different *Z. multiflora* portions (air-dried) was carried out by hydrodistillation using a Clevenger-type apparatus [21]. The extractions were carried out for 2 h and the oils were stored in dark glass bottles in a freezer until further use. GC analysis was performed using a GC (9-A Shimadzu, Japan) equipped with a flame ionization detector (FID). Quantitation was carried out on Euro Chrom 2000 software (KNAUER Company) by the area normalization method. The analysis was carried out using a DB-5 fused-silica column (30 m × 0.25 mm,

film thickness 0.25 μm) and a temperature program of 40-250°C at a rate of 4°C/min, injector temperature of 250°C, detector temperature of 265°C and the carrier gas was helium (99.99%). The GC/MS unit consisted of a Varian-3400 GC coupled to a Saturn II ion trap detector. The column of GC/MS was the same as of the GC under the same conditions that the above analysis was carried out. The constituents were identified by comparing their mass spectra with those in the computer library and with the authentic standards.

2.3 Radical-scavenging capacity (DPPH assay) of the oils

The hydrogen atom or electron donation abilities of the extracts and pure compounds were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenylpicrylhydrazyl (DPPH). This spectro-photometric assay uses the stable radical DPPH as reagent [22,23]. Among the different concentrations of essential oil preparations, 20% v/v (in methanol) was found suitable for DPPH test. Fifty μL of the essential oils in methanol was added to 5 ml of DPPH solution (0.004% DPPH in methanol). Trolox (1 mM), a stable antioxidant, was used as a synthetic reference. After 30 min of incubation period at room temperature, the absorbance was read against the blank at 517 nm. The inhibitory effects of the extracts in percent (I %) was calculated by the following formula:

$I\% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$. Where, A_{blank} is the absorbance of the control reagent (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. All the assays were carried out in triplicate (22,23).

2.4 B-Carotene-linoleic acid assay of the oils

The antioxidant activity of essential oils was determined using the β -carotene bleaching test [24]. Approximately 10 mg of β -carotene (type I synthetic) was dissolved in 10 ml of chloroform, and then 0.2 ml of this solution was added to a boiling flask containing 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator at 40°C for 10 min. Then, 50 ml of distilled water saturated with oxygen was added slowly with vigorous agitation to form an emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of essential oil solution prepared according to [25]. The absorbance was immediately measured at 470 nm against a blank consisting of an emulsion without β -carotene. The tubes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Samples containing 0.2 ml of ethanol instead of essential oils were also monitored and used as

control. Butylated hydroxytoluene (BHT 1mM in ethanol), a stable antioxidant was used as reference. The antioxidant activity was expressed as inhibition percentage with reference to the control sample after 60 min of incubation, using the following equation: $AA = 100(DR_c - DR_s)/DR_c$, where

AA = antioxidant activity %

DR_c = degradation rate of control = $[\ln(a/b)/60]$

DR_s = degradation rate in presence of sample = $[\ln(a/b)/60]$

a = absorbance at time 0

b = absorbance at 60 min

2.5 Determination of Minimal Inhibitory Concentration and Minimal Bactericidal Concentration

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were assessed according to Baron *et al.*'s (1990) [26] procedure and NCCLS M2-A7 (2000) [27]. All tests were performed in BHB. MIC, defined as the lowest concentration of the oil resulted in a complete inhibition of visible growth in the broth, was determined as follows: A bacterial suspension was made in BHB to a density of 0.5 McFarland standards and confirmed by spectrophotometrical reading at 600 nm. A standardized suspension (100 μL) of the each test organisms, separately, was inoculated in to a series of sterile tubes of BHB containing two-fold dilutions of cumin essential oils. The initial concentration of cumin essential oil in the first tube containing BHB was 1/2. This was used to prepare serial doubling dilutions over the range 25 to 0.048 mg/ml. A tube without essential oils was chosen as control. All tubes were incubated at 37°C for 24 h. Thereafter, the tubes were checked for growth of bacteria and turbidity from control tube to higher concentration [28,29]. To determine the MBC of oils, all tubes without bacterial growth were cultured on BHI and incubated at 37°C for 24h. The plates were checked for growth of bacterial colonies. MBC was evaluated as the lowest oil concentration at which no growth was observed on the plates.

2.6 Statistical analysis

Data are presented as means \pm Standard Error (SE). The results were subjected to one-way ANOVA followed by Tukey's HSD (Honestly Significant Differences) using SPSS13.0 software. The significance was determined as $P < 0.05$.

3. Results

3.1 The chemical compositions of different parts of *Z. multiflora* essential oils

Table 1. Essential oil analysis prepared from different parts of *Z. multiflora*.

No	Compound	Leaf		Flower		Aerial parts	
		%	RI	%	RI	%	RI
1	α -thujene	-	-	0.3	931.9	0.4	931.9
2	α -pinene	0.4	941.8	2.3	940.72	2.4	940.7
3	β -pinene	0.3	976.8	1.0	976.8	1.1	976.8
4	α -Terpinene	0.3	1031.1	0.7	1031.1	0.8	1031.1
5	p-cymene	2.3	1040.6	2.2	1039.3	7.6	1039.3
6	γ -Terpinene	2.6	1073.1	5.0	1073.1	4.4	1073.1
7	Thymol	8.6	1303.9	20.2	1305.2	61.8	1306.5
8	Carvacrol	72.1	1324.6	56.0	1324.6	10.6	1315.6
9	Thymyl acetate	0.2	1359.3	1.1	1359.3	2.8	1358.1
10	Geranyl acetate	2.7	1376.1	3.7	1376.1	0.5	1373.7
11	E-caryophyllene	0.4	1458.1	3.0	1458.1	2.6	1458.1
12	Aromadendrene	1.5	1477.8	1.1	1477.8	1.0	1476.3
Total		91.4		96.6		96.0	

Table 2. Composition of the essential oil of different parts of *Z. multiflora* components by chemical classes.

Compositions	Leaf	Flower	Aerial parts
Monoterpene hydrocarbones	3.6	9.3	9.1
Oxygenated monoterpene	2.7	3.7	0.5
Total monoterpenoids	6.3	13.0	9.6
Sesquiterpene hydrocarbones	1.9	4.1	3.6
Oxygenated Sesquiterpene	-	-	-
Total sesquiterpenoids	1.9	4.1	3.6
Phenolic compounds	80.7	76.2	72.4
Ester	2.9	4.8	3.3
Aromatic hydrocarbone	2.3	2.2	7.6
Total hydrocarbons	7.8	14.6	20.3
Total oxygenated compounds	83.6	81.0	75.7

The oil extraction yields from the leaf, flower and aerial parts were ~ 1.07, 2.5 and 3 % (w/w), respectively. GC/GC-MS analysis of the essential oils derived from three samples resulted in identification of 12 known compounds (Table 1). As shown, the main components of three parts including Leaf, flower and aerial parts were carvacrol and thymol. In addition, the compositions of the essential oil of different parts of *Z. multiflora* components by chemical classes are demonstrated in Table 2. As shown, the total oxygenated and phenolic compounds were identified as the major classes of the chemical compounds of essential oils in all parts (Table 2).

3.2 The antioxidant activities of different parts of *Z. multiflora* essential oils

The antioxidant properties of the different parts of *Z. multiflora* essential oils analyzed by DPPH and β -carotene bleaching tests are presented in Figure

1&2. When compared to a standard antioxidant agent *i.e.* trolox (15%), it was found that the essential oils extracted from different parts of *Z. multiflora* had strong radical scavenging activity (~68%). Addition of the plant oils to the reaction mixture containing β -carotene and linoleic acid, brought about ~95% inhibition in formation of peroxidation products that was equal in all plant parts (Fig. 2).

The radical scavenging activity of the oil samples were measured spectrophotometrically using DPPH free radical. Trolox was used as positive control. Results are mean \pm SEM of three analyses carried out on essential oils derived from fresh and irradiated *Z. multiflora* Boiss. The antioxidant activity of the *Z. multiflora* oil samples were measured spectrophotometrically using β -carotene bleaching test. BHT was used as positive control. Results are mean \pm SEM of three analyses carried out on essential oils derived from fresh and irradiated *Z. multiflora* Boiss.

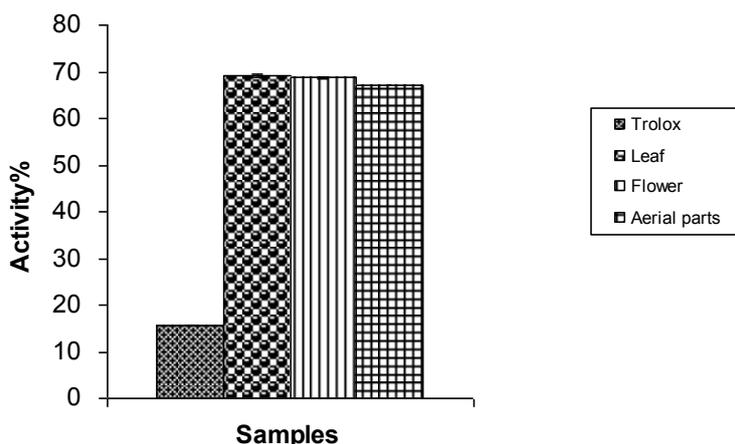


Figure 1. Free radical scavenging activities (%) of *Z. multiflora* essential oils.

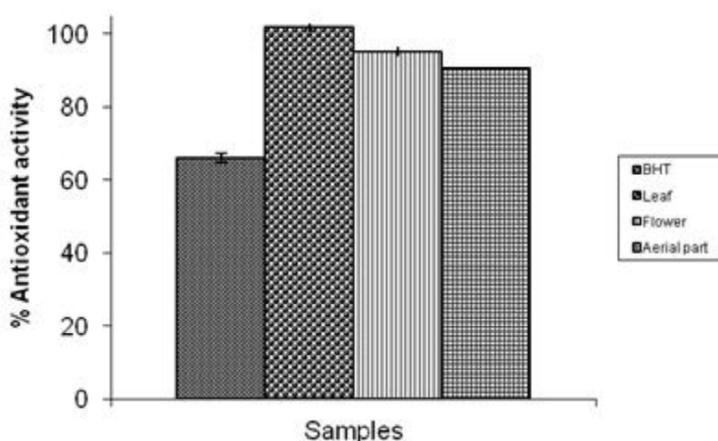


Figure 2. Antioxidant activities (%) of *Z. multiflora* essential oils.

Table 3. MIC and MBC determinations of the essential oils (mg/mL).

Microorganisms	Leaf		Flower		Aerial part	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Bacillus cereus</i>	2.6 ± 0.52	2.6 ± 0.52	3.1 ± 0.00	4.16 ± 1.04	2.6 ± 0.52	3.12 ± 0.00
<i>Staphylococcus aureus</i>	2.08 ± 0.52	2.08 ± 0.52	2.6 ± 0.52	2.6 ± 0.52	2.6 ± 0.52	2.6 ± 0.52
<i>Escherichia coli</i>	0.65 ± 0.13*	0.65 ± 0.13*	0.13 ± 0.03#*	0.13 ± 0.03#*	0.26 ± 0.06#*	0.65 ± 0.13*
<i>Pseudomonas aeruginosa</i>	1.56 ± 0	1.56 ± 0	0.26 ± 0.06#*	0.52 ± 0.13#*	0.32 ± 0.06#*	0.52 ± 0.13#*

3.3 The antimicrobial activities of different parts of *Z. multiflora* essential oils

The results of MIC and MBC methods showed that *E. coli* as well as *P. aeruginosa* as Gram-negative bacteria ($P > 0.05$), is more sensitive to the effects of the oils derived from leaf, flower and aerial parts as compared to *B. cereus* and *S. aureus* as Gram-positive bacteria ($P < 0.05$) (Table 3).

Data are mean ± S.E.M. of three different experiments. (*) denote significantly more sensitive from the non-marked groups in each column ($P < 0.05$). (#) denote

significantly more sensitive from the same group in the leaf ($P < 0.05$).

4. Discussion

Our results revealed that despite the minor changing in the individual oil constitutes, the main components are the same in different parts of the plant. The major compositions containing carvacrol and thymol in the oils from three parts (leaf, flower and aerial parts) of *Z. multiflora* (Table, 1) were in similarity with other studies [2,3,12,30-32]. Indeed, the oils isolated from different parts of *Z. multiflora* were found to be rich in oxygenated and phenolic compounds (Table. 2). These results are agreement with previous studies

that reported the most abundant in *Z. multiflora* essential oils as oxygenated monoterpenes (approximately 70%) [4,33]. Gupta et al. reported that the essential oil of the Indian ecotype consists of 69% phenols containing mainly carvacrol [34].

Our data indicated that the essential oils derived from different parts of *Z. multiflora* i.e. leaf, flower and aerial parts had the equal radical scavenging and antioxidant properties of the different parts of the oils (Figs. 1&2). Other studies confirmed these results indicated that the essential oil of *Z. multiflora* possessed antioxidant activities as measured by the DPPH free radical scavenging and beta-carotene bleaching assays [2,4,35]. Another study also showed the inhibitory effects of carvacrol and thymol derived from *Z. multiflora* aerial parts on nitric oxide and hydrogen peroxide production in glucose-stimulated human monocyte [32].

The MIC and MBC of the oils derived from leaf, flower and aerial parts of *Z. multiflora* ranged from 0.5 to 4 mg/mL, indicating the sensitivity of all microorganisms e.g. *E. coli*, *P. aeruginosa*, *B. cereus* and *S. aureus* to the effects of the oils in different degree (Table 3). These data are in agreement with other studies indicating that the essential oils derived from aerial parts of *Z. multiflora* entirely inhibited the growth of yeasts at concentrations of less than 1 μ L/mL. Moreover, the oils exhibited significant bacteriostatic and bactericidal activities against Gram-positive and Gram-negative bacteria at concentrations ranging from 0.12 to 8 μ L/ML. [36] The anti-microbial activity of most essential oils is related to their phenolic monoterpenes (4). *Z. multiflora* essential oil, with a very high percentage of thymol and carvacrol, possess significant anti-microbial activity [37]. Electron micrographs showed that these compounds, which are lipophilic in nature, do something on the cell membrane and cause considerable morphological damage, leading to change in permeability and the release of cellular contents [38].

In comparing the effects of three oil samples, it can be deduced that the *E. coli* is more sensitive to the oils derived from flower and aerial parts ($P>0.05$) rather than the leaf oil ($P<0.05$). Also, the *P. aeruginosa* is more sensitive to the oils derived from flower and aerial parts ($P>0.05$) rather than leaf oil ($P<0.05$). While, the *S. aureus* and *B. cereus* are equally sensitive to the oils derived from leaf, flower and aerial parts ($P>0.05$).

5. Conclusions

It can be deduced that the essential oils derived from leaf, flower and aerial parts of the *Z. multiflora* are fine sources of oxygenated monoterpenes, in particular thymol and carvacrol, with considerable antioxidant and antimicrobial properties. Regarding

to the biological properties, these extracts can be suitable candidates for medicinal uses.

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